

(b) incubating the compound and the composition under conditions promoting cell growth; and

(c) observing the cells for any effect not observed in cells not brought into contact with the composition.

Remarks

Claims 1-6, 8-22, and 24-32 would be pending upon entrance of this Amendment. Amendments to claims 1, 5, 6, 8, 13, 21, 22, 24, 31, and 32 are proposed and claims 7 and 23 are cancelled with this Amendment. A copy of all pending claims as they would be amended upon entry of this amendment is attached to this Amendment and Response in an Appendix.

Amendments to the Claims

The claims have been amended to more clearly define the claimed composition as including a substrate having tethered thereto an effective concentration of one or more growth effector molecules to enhance the rate of target cell growth over the rate of target cell growth with soluble growth effector molecules and growth effector molecules merely adsorbed to a substrate, wherein the tethers are water soluble and branched and are able to covalently link more than one growth effector molecule.

Support for the aspect of water solubility is found in the specification at page 6, line 24; page 7, line 2 and previously pending claim 7.

Support for the aspect of the tethers being branched and being able to covalently link more than one growth effector molecule is found in the specification at page 4, lines 17-18, page 7, lines 3-9, and page 12, lines 25-28.

Support for the aspect of growth effector molecules attached to the substrate in a concentration effective to enhance the rate of target cell growth over the rate of target cell growth with soluble growth effector molecules is found at page 4, lines 11-16 and in the Figures. Support for the aspect of growth effector molecules attached to the substrate in a concentration effective to enhance the rate of target cell growth over the rate of target cell growth with growth effector molecules merely adsorbed to a substrate is found at page 24, lines 18-22 and in Figure 2.

The Advisory Action

The Advisory Action refused entry of the amendments in the response mailed July 14, 1997, on the basis that the amendment raised new issues that would require further consideration and/or search and issues of new matter. The amendment to the specification of the term "ingestible" is not repeated in the present response. Claims 7 and 23 are herein expressly cancelled. The Examiner indicated in the Interview that the proposed drawing change has been approved.

The Tethers Bind More Than One Factor

To clarify how the claimed compositions have tethers able to covalently link more than one factor, the term "branched" has been added to the claims to describe the tethers. It

is submitted that the aspect of the tethers being branched and able to covalently link more than one growth effector molecule is not a new issue. The claims as originally filed encompassed both branched and linear tethers and the search would have encompassed both. Moreover, the disclosure of branched tethers is supported in the specification at, for example, page 4, lines 16-18 where it is disclosed that "In a preferred embodiment, multiple growth factors and/or matrix materials are attached to a single core molecule, such as a star polymer." The Examiner has searched for, and cited against the claims, art disclosing branched polymers. Therefore, this is not a new issue.

The Phrase "Enhance The Rate Of Cell Growth"

In response to the rejection under 35 USC §112, second paragraph, with respect to the phrase "enhance the rate of cell growth", the claims are amended to recite that the growth effector molecules are attached to the substrate in a concentration effective to enhance the rate of target cell growth over the rate of target cell growth with soluble growth effector molecules and growth effector molecules adsorbed to a substrate. This amendment is supported at page 4, lines 11-16 and at page 5, lines 16-30 and by the Figures and should overcome the rejection.

Rejections under 35 U.S.C. §112 in the Office Action of April 14, 1997

Claims 1-32 were rejected under 35 U.S.C. §112, second paragraph, and claim 8 was rejected under 35 U.S.C. §112, fourth paragraph. Claims 5, 6, 21, and 22 have been

amended to clarify that it is the substrate polymer that is referred to in the claims. Claim 8 has been amended to delete "starch" as a synthetic polymer.

Rejections under 35 U.S.C. §102 and/or §103 in the Office Action of April 14, 1997

Clapper

Claims 1-9, 13, 18-25, and 31 were rejected under 35 U.S.C. §102(b) as disclosed by U.S. Patent No. 5,512,424 to Clapper et al. ("Clapper"). Claims 10-12 and 26-28 were rejected under 35 U.S.C. §103(a) over Clapper. This rejection is respectfully traversed if applied to the amended claims.

Clapper describes the use of a combination of a cell adhesion factor and a positively charged molecule, each bound to the surface of a cell culture support, to promote cell adhesion to the support, in order to culture adhesion- or attachment-dependent cells. Clapper teaches that the use of the combination of a positively charged molecule and a cell adhesion factor is advantageous because the cells can attach through both receptor-mediated attachment, i.e. by interaction with the cell adhesion factor, and also non-receptor-mediated, i.e. charge-related adhesion, through interaction of the negatively charged cell constituents and the positively charged molecules. See Clapper column 6, lines 25-31. Clapper encourages cell adhesion to the positively-charged polymers. The positively charged polymers include carboxymethylcellulose, as noted by the Examiner, but the carboxymethylcellulose has been modified by the addition of positively charged groups. See Clapper column 7, lines 24 column 8, line 2 and claim 1.

In the claimed compositions and methods, on the other hand, tethers are made out of polymers which are water soluble. This is desirable in Applicants' compositions and methods to provide the tethered growth factors with flexibility and substantial mobility, a characteristic which is deemed critical in Applicants' compositions and methods. See the specification, page 6, lines 13-26 ("Substantial mobility of a tethered growth factor is critical"). The claims have been amended to reflect these characteristics of the tether.

Moreover, Clapper does not disclose, or lead one to believe, that cells attached to the compositions disclosed therein have, or could have, an enhanced rate of growth over the rate of cell growth with soluble or adsorbed growth effector molecules. Clapper also does not disclose that the tethers are able to bind more than one growth effector molecule.

Herweck et al. in view of Merrill

Claims 1-9, 13-16, 18-25 and 31 were rejected under §103 as obvious over U.S. Patent No. 5,370,681 to Herweck et al. ("Herweck"), in combination with U.S. Patent No. 5,171,264 to Merrill ("Merrill '264"). This rejection is respectfully traversed if applied to the amended claims.

As the Examiner notes, Herweck does not teach biocompatible tethers which have one end covalently linked to a substrate and a growth effector molecule covalently linked to the other end. Nor does Herweck contain any suggestion for incorporating such a tether. Merrill '264 discloses immobilized star PEO molecules "that can be used as a tool for separating and purifying biological molecules". There is no suggestion for binding growth

effector molecules to the PEO molecules or for using the PEO molecules in cell growth applications. Therefore, there is no motivation to combine the references, as required.

"There must be some reason, suggestion, or motivation found in the prior art whereby a person of ordinary skill in the field of the invention would make the combination." *In re Oetiker*, 24 USPQ2d 1443 (Fed. Cir. 1992)

There is no teaching or suggestion in either Herweck or Merrill '264 that would lead one of skill in the art to make at a minimum the following alterations: select bioactive molecules **enhancing** growth rate in the amount required to enhance growth rate when not internalized and to chemically couple the molecules to the substrate in a density and with appropriate linkers to result in enhanced growth rates of attached cells over the growth rate of cells exposed to soluble or adsorbed growth effector molecules.

Herweck in view of Merrill '264 and Merrill

Claims 10-12 and 26-28 were rejected under §103 as obvious over Herweck in view of Merrill '264 and further in view of Merrill, J. Biomater. Sci. Polymer. 5, 1-11 (1993). This rejection is respectfully traversed if applied to the amended claims.

Merrill (1993) also does not contain any suggestions to attach growth effector molecules to PEO tethers in an amount effective to enhance the growth rate of cells when not internalized and to chemically couple the molecules to the substrate in a density and with appropriate linkers to result in enhanced growth rates of attached cells. Therefore, there is

no motivation to combine the references. Moreover, even if the references were to be combined, the combination would not yield the claimed compositions and methods.

Herweck in view of Merrill '264 and Mikos

Claim 17 was rejected under §103 as obvious over Herweck, in combination with Merrill '264 in combination with U.S. Patent No. 5,522,895 to Mikos et al. ("Mikos"). This rejection is respectfully traversed if applied to the amended claims. Claim 17 is dependent upon claim 13 which, as discussed above, is not disclosed or made obvious by Herweck and Merrill '264. Mikos does not add the elements missing from the Herweck/ Merrill combination.

Mikos is similar to Herweck in that it is directed to a matrix for seeding with cells that can be implanted. It also does not disclose or make obvious selecting bioactive molecules enhancing growth rate, determining the amount required to enhance growth rate when not internalized, and to chemically couple the molecules to the substrate in a density and with appropriate linkers to result in enhanced growth rates of attached cells.

Herweck in view of Merrill '264 and Naughton

Claims 29 and 32 were rejected under §103 as obvious over Herweck in combination with Merrill '264 and U.S. Patent No. 5,032,508 to Naughton et al. ("Naughton"). This rejection is respectfully traversed if applied to the amended claims.

Naughton is actually quite similar to Clapper. It discloses a matrix which might be suitable for implantation, having attached thereto stromal cells that serve as attachment factors for other types of cells grown on the matrix. One skilled in the art would be led by the disclosure of Naughton to believe that no further modifications were necessary in order to grow cells since the stromal cells reportedly result in adequate cell attachment and growth. Moreover, Naughton also does not contain any suggestions to combine the individual teachings of Herweck and Merrill and does not disclose or make obvious selecting bioactive molecules **enhancing** growth rate, determining the amount required to enhance growth rate when not internalized, and to chemically couple the molecules to the substrate in a density and with appropriate linkers to result in enhanced growth rates of attached cells.

Herweck in view of Merrill '264 and Tomomura

Claims 29 and 30 were rejected over Herweck in combination with Merrill '264 and Tomomura et al. J. Cell. Physiol. 30:221-227 (1987). This rejection is respectfully traversed if applied to the amended claims. Tomomura also does not suggest combining the individual teachings of Herweck and Merrill and does not disclose or make obvious selecting bioactive molecules **enhancing** growth rate, determining the concentration and density of coupled growth factors required to enhance growth rate when not internalized, and to chemically couple the molecules to the substrate in a density and with appropriate linkers to result in enhanced growth rates of attached cells.

European Patent Application 531733

Claims 1-7, 9, 10, 13, 18-21, 23, 25, 26, 29-31 were rejected under 35 U.S.C. §102(b) as being anticipated by European Patent Application 531733 ("EP '733"). Claims 10-12 and 26-28 were rejected under 35 U.S.C. §103(a) over EP '733. These rejections are respectfully traversed if applied to the amended claims.

EP '733 discloses a carrier to which is immobilized a cell growth factor. The factor is attached to the carrier through a linker or spacer. The spacer is preferably about 2 nm in length (page 3, line 53) and is a polymer compound such as polyethyleneimine, polyamino acid or polymethylene (page 3, lines 56-58). It is stated that the presence of a cationic group on the spacer molecule is desirable to aid in cell adsorption to the surface through electrostatic force (page 4, lines 1-3). EP '733 does not teach or suggest a cell growth factor "tethered" to a substrate as claimed by Applicants but rather discloses a cell growth factor "immobilized" on a substrate (see claim 1 and page 2, lines 8 and 48). Thus EP '733 discloses a composition similar to the adsorbed cell growth factor compositions to which Applicants compare their tethered compositions. See Figure 2 and the discussion at page 24, where it is demonstrated that Applicants' tethered growth effector molecules enhance cell growth as compared to adsorbed growth effector molecules.

The polymers that EP '733 teaches, for example, polyethyleneimine, are not soluble in aqueous solution and will bunch up in aqueous solution, making the spacers have an effective length of about 2 nm (see page 3, line 53). The polymers of the claimed compositions and methods, on the other hand, are very soluble in aqueous solution and will

extend to their full length, providing a wide range of movement (flexibility) to the factors attached thereto. This is a very important aspect of Applicants' tethers. As discussed in the application at page 6, lines 6-8 and 11-26 and page 7, lines 21-30, the tether must be flexible to allow the growth factor to contact the receptor and also to allow the growth factor-receptor complex mobility within the cell membrane. (See, for example, page 6, line 19, "Substantial mobility of a tethered growth factor is critical") EP '733 thus teaches away from the Applicants' invention, because it teaches use of non-water soluble polymers that will interact with the cell.

EP '733 does not disclose that the compositions taught therein enhance the rate of cell growth over the rate of cell growth due to soluble or adsorbed growth effector molecules. EP '733 does not disclose a comparison of cell growth with its immobilized factors with soluble or adsorbed factors and it is doubtful that results similar to Applicants' would be obtained.

WO 89/05616

Claims 1-10, 12-26, 28, and 31 were rejected under 35 U.S.C. §102(b) as being anticipated by WO 89/05616 by Bio-Metric Systems, Inc. ("WO '616"). Claims 11 and 27 were rejected under 35 U.S.C. §103(a) over WO '616. These rejections are respectfully traversed if applied to the amended claims.

WO '616 discloses linear polymeric tethers having one end attached to a support and the second end attached to a biomolecule useful in cell culture. WO '616 discloses that the

tethers are heterobifunctional, having two reactive groups thereon, one which binds to the substrate and one which binds to the biomolecule. In other words, WO '616 does not teach or suggest that the tethers can bind more than one biomolecule, as claimed by Applicants. This is an important aspect of Applicants' claimed compositions and methods, as discussed in the application on page 7, lines 3-8 and page 12, lines 25-28. Applicants' tethers can bind more than one molecule of the same growth effector or can bind different growth effector molecules. Thus, the density of a growth effector molecule on a substrate can be increased without substantially increasing the number of cell-repellant tethers. Alternatively, for example, both insulin and EGF could be tethered to the same substrate, allowing presentation of both molecules to the cell.

Under the approach outlined in the WO '616, in theory any concentration of molecules could be attached. However, since only linear tethers, i.e. tethers with only one attachment site for a factor and one attachment site to the substrate, are used, going to lower concentrations also increases the distance between factors and potentially inhibits the ability of receptor-factor complexes to interact in the cell membrane. Thus, at lower concentrations, signalling may not occur at all using linear tethers, because the factors are homogeneously spaced on the surface. By using a multi-functional tether, Applicants can go to very low factor concentrations and still allow receptor aggregation by virtue of having more than one factor on each tether. So, even though the tethers can be very far apart (i.e. the distance from the center of one tether to the center of the adjacent tether is more than

twice the fully extended chain length of the tether). receptor-receptor interactions can still occur in the membrane after ligand-binding because the factors are locally clustered.

WO '616 does disclose use of tethers to attach biomolecules to support surfaces. Among the biomolecules mentioned are some of the growth effector molecules claimed by Applicants. However, the only examples in WO '616 involving cells also involved tethering of collagen, hyaluronic acid, and fibronectin (Examples 3 and 6). WO '616 did not report enhanced growth of cells but only enhanced adhesion. Therefore, it is not surprising that the compositions and methods of WO '616 result in increased cell adhesion.

EGF itself attenuates cell adhesion, in other words, cells may spread out on the substrate in the absence of EGF (i.e., they may adhere and spread on a substrate that bears a "high" tether concentration resulting in a "low" tether spacing, a spacing less than two times the radius of gyration), but round up (become less adherent) in its presence. Rounded cells generally do not undergo DNA synthesis in culture and in fact they may round up so much in the presence of a tethered factor that they do not adhere at all and undergo apoptosis (programmed cell death that occurs in the absence of sufficient adhesion). WO '616 does not teach how to tether EGF to alter cell growth. WO '616 teaches the use of PEO (a polymer commonly grafted to surfaces to inhibit cell adhesion), and generally teaches high concentrations of tether with no specifics about how to cause cell adhesion in the presence of these types of tethers and avoid non-adherent cells. On the other hand, Applicants show how to enhance the rate of cell growth as compared to the rate of cell growth with soluble or adsorbed molecules by balancing use of polymeric water soluble tethers which do not bind to

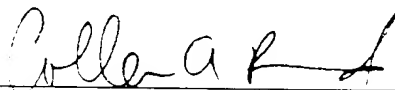
cells and the use of the proper amounts of tethered growth effector molecules. WO '616 teaches how to tether proteins, but not how to tether EGF so that cell growth will be enhanced.

In summary, the claims have been amended to more clearly define novel and non-obvious aspects of the claimed compositions and methods. The cited prior art references do not teach or suggest compositions or methods for enhancing cell growth involving the use of a water soluble polymeric tether attached to a substrate and able to bind more than one growth effector molecules so that the molecules cannot be internalized by the cell and the growth of target cells is enhanced as compared to the rate of cell growth of cells exposed to soluble and adsorbed growth effector molecules.

Indeed, it is surprising that Applicants obtained the results observed, because there was as great a likelihood that the coupled growth factors would sterically hinder binding of the growth factors to the cell, actually decreasing the effectiveness of the growth factors on the cell growth rate, as there was that the same, much less enhanced, rate of growth would be observed when growth factors were administered in soluble form or coupled singly to the substrate. Nowhere has the Examiner pointed to any literature that would indicate that one skilled in the art would predict that the claimed compositions would be effective to enhance cell growth rate.

Allowance of all claims 1-6, 8-22, and 24-32, as amended, is earnestly solicited.

Respectfully submitted,



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Dated: September 3, 1997

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APPENDIX: Claims as pending after entry of the Amendment

1. (twice amended) A composition for stimulating the growth of eukaryotic cells comprising
a biocompatible solid substrate,
biocompatible synthetic branched water soluble polymeric tethers, and
growth effector molecules,
wherein one end of each tether is covalently linked to the substrate and each growth effector molecule is covalently linked to a distal end of a tether so that the growth effector molecule cannot be internalized by cells attached to the substrate, each tether is able to covalently link more than one growth effector molecule, and
the growth effector molecules are attached to the substrate in a concentration effective to enhance the rate of target cell growth over the rate of target cell growth with soluble growth effector molecules and growth effector molecules adsorbed to a substrate without internalization of the molecules.
2. The composition of claim 1 wherein the form of the biocompatible substrate is selected from the group consisting of netting, individual and woven fibers, sponge and shaped polymers.
3. The composition of claim 2 wherein the shape of the shaped polymer is selected from the group consisting of dishes, bottles, solid particles, hollow particles, and polymers shaped to match a desired tissue shape.
4. The composition of claim 1 wherein the biocompatible substrate is selected from the group consisting of glasses, metals and biocompatible polymers.
5. (twice amended) The composition of claim 4 wherein the substrate polymer is selected from the group consisting of synthetic polymers and natural polymers.
6. (twice amended) The composition of claim 5 wherein the substrate polymer is selected from the group consisting of proteins, polysaccharides, polyesters, polycaprolactone, polyhydroxybutyrate, polyanhydrides, polyphosphazenes, polyorthoesters, polyurethanes, and combinations thereof.
8. (amended) The composition of claim 1 wherein the tether is selected from the group consisting of polyethylene oxide and carboxymethylcellulose.

9. The composition of claim 1 wherein the growth effector molecules are selected from the group consisting of epidermal growth factor, platelet-derived growth factor, transforming growth factor, hepatocyte growth factor, heparin binding factor, insulin-like growth factor I or II, fibroblast growth factor, erythropoietin, nerve growth factor, bone morphogenic proteins, muscle morphogenic proteins, extracellular matrix molecules, and combinations thereof.

10. The composition of claim 1 wherein the tether has a backbone length between 5 and 50,000 atoms.

11. The composition of claim 10 wherein the tether has a backbone length between 100 and 50,000 atoms.

12. The composition of claim 10 wherein the tether has a backbone length between 5 and 500 atoms.

13. (twice amended) A method for growing eukaryotic cells comprising
(a) bringing into contact the cells and a composition comprising
a biocompatible solid substrate,
biocompatible branched water soluble polymeric tethers, and
growth effector molecules,
wherein one end of each tether is covalently linked to the substrate, each
tether is able to covalently link more than one growth effector molecule,
each growth effector molecule is covalently linked to a distal end of a
tether so that the growth effector molecule cannot be internalized by cells attached
to the substrate, and
the growth effector molecules are attached to the substrate in a
concentration effective to enhance the rate of target cell growth over the rate of
target cell growth with soluble growth effector molecules and growth effector
molecules adsorbed to a substrate, without internalization of the molecules; and
(b) maintaining the contacting cells and composition under conditions and for a time
sufficient to cause the cells to grow.

14. The method of claim 13 wherein the step of bringing into contact comprises
administering the composition to a patient in need of cell growth.

15. The method of claim 14 wherein the composition is administered by injection,
infusion, or implantation.

16. The method of claim 15 wherein the composition is administered by implantation
of the composition and wherein the substrate is shaped to match a desired tissue shape.

17. The method of claim 16 wherein the substrate is biodegradable.
18. The method of claim 13 wherein the form of the biocompatible substrate is selected from the group consisting of netting, individual and woven fibers, sponges and shaped polymers.
19. The method of claim 18 wherein the shape of the shaped polymer is selected from the group consisting of dishes, bottles, solid particles, hollow particles, and polymers shaped to match a desired tissue shape.
20. The method of claim 13 wherein the biocompatible substrate is selected from the group consisting of glasses and biocompatible polymers.
21. (amended) The method of claim 20 wherein the substrate polymer is selected from the group consisting of synthetic polymers and natural polymers
22. (amended) The method of claim 21 wherein the substrate polymer is selected from the group consisting of polylactic acid, polyglycolic acid, polyanhydrides, polyorthoesters, collagen, glycosaminoglycans, polyamino acids, and combinations thereof.
24. (amended) The method of claim 13 wherein the tether is selected from the group consisting of polyethylene oxide, carboxymethylcellulose, and starch.
25. The method of claim 13 wherein the growth effector molecules are selected from the group consisting of epidermal growth factor, platelet-derived growth factor, transforming growth factor, hepatocyte growth factor, heparin binding factor, insulin-like growth factor I or II, fibroblast growth factor, erythropoietin, nerve growth factor, bone morphogenic proteins, muscle morphogenic proteins, extracellular matrix molecules, and combinations thereof.
26. The method of claim 13 wherein the tether has a backbone length between 5 and 50,000 atoms.
27. The method of claim 26 wherein the tether has a backbone length between 100 and 50,000 atoms.
28. The method of claim 13 wherein the tether has a backbone length between 5 and 500 atoms.
29. The method of claim 13 wherein the cells are selected from the group consisting of parenchymal cells and stem cells.

30. The method of claim 29 wherein the cells are hepatocytes.
31. (twice amended) A cell culture comprising
a biocompatible solid substrate,
biocompatible branched water soluble polymeric tethers,
growth effector molecules, and
growing cells,
wherein one end of each tether is covalently linked to the substrate, each tether
is able to covalently link more than one growth effector molecule,
each growth effector molecule is covalently linked to a distal end of a tether so
that the growth effector molecule cannot be internalized by cells attached to the substrate,
the growth effector molecules are attached to the substrate in a concentration
effective to enhance the rate of target cell growth over the rate of target cell growth with
soluble growth effector molecules and growth effector molecules adsorbed to a substrate,
without internalization of the molecules, and wherein the growing cells are bound to the
growth effector molecules.
32. (twice amended) A method of testing a compound for an effect on tissue
comprising
(a) bringing into contact the compound to be tested and a composition comprising
a biocompatible solid substrate,
biocompatible branched water soluble polymeric tethers,
growth effector molecules, and
growing cells,
wherein one end of each tether is covalently linked to the substrate, each
tether is able to covalently link more than one growth effector molecule,
each growth effector molecule is covalently linked to a distal end of a
tether so that the growth effector molecule cannot be internalized by cells attached
to the substrate,
the growth effector molecules are attached to the substrate in a
concentration effective to enhance the rate of target cell growth over the rate of
target cell growth with soluble growth effector molecules and growth effector
molecules adsorbed to a substrate, without internalization of the molecules, and
wherein the growing cells are bound to the growth effector molecules;
(b) incubating the compound and the composition under conditions promoting cell growth;
and
(c) observing the cells for any effect not observed in cells not brought into contact with
the composition.